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EXPERIMENTAL ARENA FOR CONFINING THRIPS AND OTHER SMALL ARTHROPODS IN THE LABORATORY

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Abstract.—A new experimental arena to confine western flower thrips, *Frankliniella occidentalis* (Pergande) and other small arthropods in order to conduct laboratory bioassays and behavioral studies is described. Arenas are constructed from cardboard paper cartons using a clear plastic petri dish lid and weather stripping to form an insect tight seal. A water pic inserted into the side of the arena is used to maintain plant material within the arena. The configuration of the arenas allows for direct visual observation of arthropods on portions of whole plants using a dissection microscope while leaving the arena sealed. The arenas successfully confined western flower thrips adults for 14 d and survival rates in the arenas over 7 d were at levels acceptable for check treatments in laboratory bioassays.

Key Words.—Insecta, experimental arena, bioassay, *Frankliniella occidentalis*

Thrips are a major pest of ornamental and food crops and their pest status seems to be increasing on a wide range of crops (Mound & Teulon 1995). Plants are damaged by direct feeding on foliage, fruit and flowers and by thrips vectored disease. Management strategies have relied almost exclusively on repeated chemical applications. Intensive use of chemical controls has resulted in widespread development of resistance by economically important species such as the western flower thrips, *Frankliniella occidentalis* (Pergande) (Immaraju et al. 1992, Robb et al. 1995). As a result, there has been an increased interest in the development of alternative control agents such as predators, parasitoids and pathogens for thrips management in addition to evaluation of new biorational pesticides for thrips control.

The small size and intense activity of thrips, particularly adults, has made confinement and treatment in experimental arenas for laboratory bioassays challenging. Most researchers have relied on Munger cells (Munger 1942) or modifications thereof (Tashiro 1967, Morse et al. 1986) to conduct bioassays of chemical pesticides against thrips and other small arthropods. Typically, a known number of arthropods are confined on a leaf surface within the cells, exposed to a chemical pesticide and monitored for mortality over time. However, use of Munger cells has several disadvantages: their small size allows only a few insects to be assayed for extended periods of time; maintaining leaf vigor during the experiment can limit the length of bioassays; direct application of chemical treatments to thrips and other arthropods can be difficult; efficacy can only be determined in a simplified environment (a flat leaf surface); and realistic temperature and relative humidity conditions are difficult to manipulate within the cells. Nonetheless, use of Munger cells has proven to be a useful standard technique for evaluating pesticides against thrips and other small arthropods.

The limitations of Munger cells reduces their usefulness for evaluations of alternative control agents such as microbial insecticides, natural enemies and some biorational insecticides which act more slowly than conventional pesticides and therefore require longer observational times. Maintaining plant vigor through this

interval is critical. Thus, bioassays of alternative agents conducted on simple leaf surfaces in small enclosed cells may not simulate actual mortality in a field setting occurring over a 2 week time period and within complex environments.

Here we describe an alternative experimental arena used to evaluate the performance of entomopathogenic fungi against western flower thrips, *Frankliniella occidentalis.* Bioassays using entomopathogenic fungi can take 7 d or more and the degree of effectiveness can be influenced by temperature and relative humidity conditions. Furthermore, the behavior of thrips on plants may determine the degree of exposure to the pathogen and influence the extent of fungal infection and mortality. Therefore, we designed arenas with greater volume that can enclose larger amounts of plant material (whole leaves, terminal shoots, buds and flowers). This allowed assays of larger numbers of thrips in a situation similar to that in which thrips are found in the field or greenhouse while minimizing influence of the experiment on thrips behavior. Through ventilation of the containers we also achieved greater control over internal environmental conditions. As a result, experiments using these arenas are able to assess the efficacy of fungi on a wide range of different plant parts and under temperature and relative humidity regimes that more closely simulate field conditions.

MATERIALS AND METHODS

Construction.—Components of an experimental arena are shown in Fig. 1. Units are constructed using a pint (473 ml) paper can (Fonda Paper can, Fonda Group, P.O. Box 500953, St Louis, MO) cut to a height of 5.1 cm (Fig. 1a). Two 2.5 cm holes are cut in the sides of the paper can and covered with organdy polyester netting to allow for airflow. The top of the arena uses a 90 mm diameter plastic petri dish lid (100 × 15 mm petri dish, Fisher Scientific, Pittsburgh PA) lined with foam weather stripping (Fig, 1b). Two rubber bands compress the lid to the top of the paper can to form a seal preventing thrips from escaping. A 5 ml plastic specimen tube (Rohre tube, Sarstedt, Germany) is used as a water pic to support plant material (Fig. 1c). The cap of the specimen tube is mounted into the side of the paper can by cutting a hole just large enough so the cap can be inserted through the can and sealed using silicon glue. The tube can then be inserted into the cap mounted into the side of the can which operates as a water reservoir (Fig. 1d). A stem, branch or petiole can be inserted through a hole drilled in the cap on the inside of the arena. Replenishing water from the outside is inserted through a water hole drilled in the tube.

Evaluation.—The experimental arenas were evaluated for their ability to 1) successfully confine thrips for 14 d without escape or significant increases in adult mortality, 2) demonstrate and quantify variation in survival rates of adult thrips on rose foliage in the arenas over a 7 d period, and 3) monitor the internal temperature and relative humidity to determine the influence of arenas on environmental conditions. Adult thrips of various ages were collected from a thrips colony maintained in a greenhouse on potted chrysanthemums. For the confinement test 43 to 54 adults were placed in each of seven arenas, 4 arenas contained rose foliage and 3 contained chrysanthemum foliage. The arenas were maintained at 26° C and 60% RH in an environmental chamber during the course of the tests. For the survival tests 23 to 53 adults were placed in arenas containing rose foliage and held at 26° C and 75% RH for 7 d. The tests compared thrips survival rates

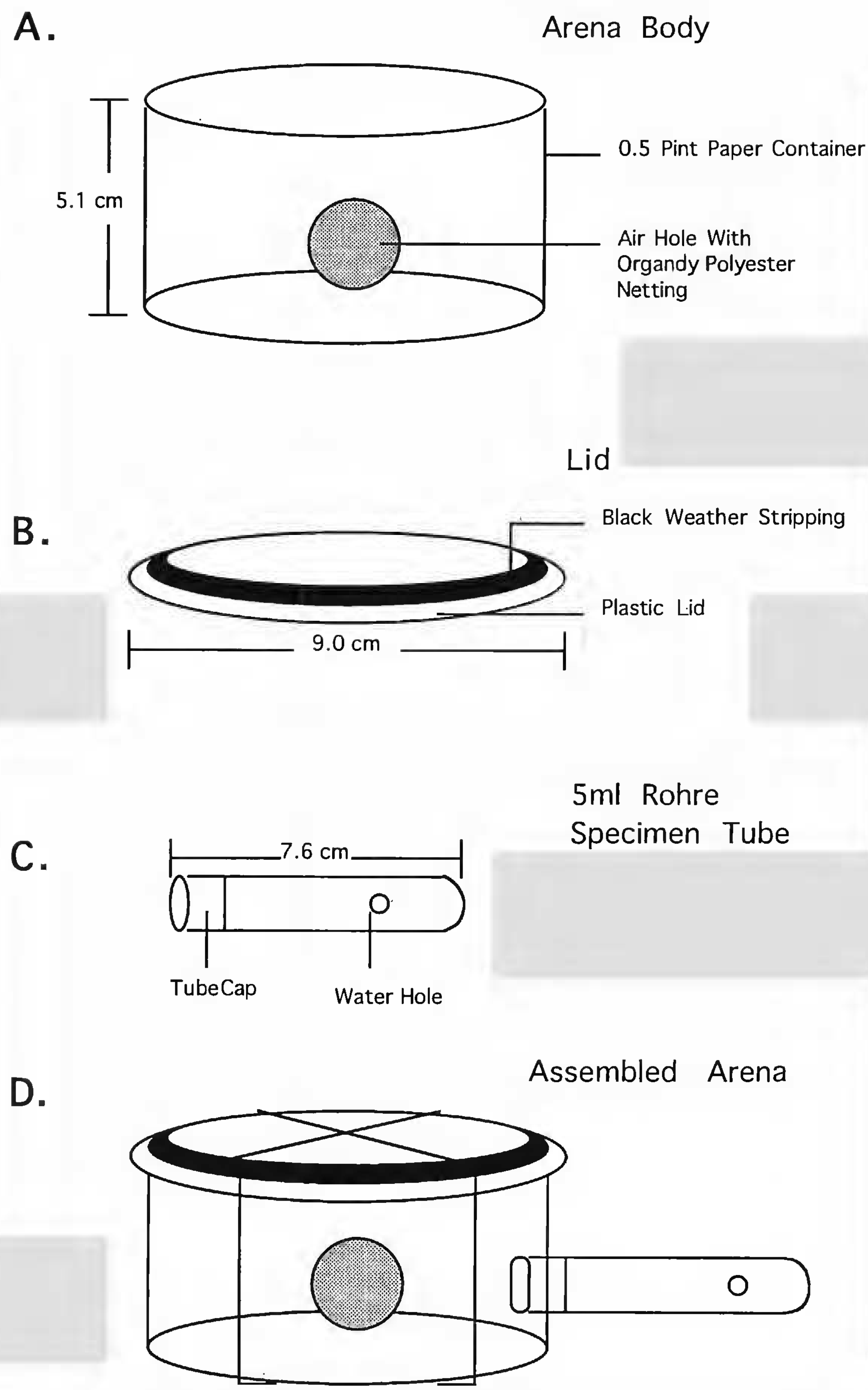

Figure 1. Schematic of experimental arena components, a) arena body, b) lid, c) specimen tube and d) assembled arena.

among five arenas within each experiment and 3 different experiments were performed on different dates. At the end of the experiments all thrips were removed from the arenas and the number of live and dead thrips recorded. Two other arenas containing plant material but no thrips were used to compare the influence of arenas on the temperature and relative humidity within the containers using a digital temperature and relative humidity meter (Fisher scientific, model 1055712). The expected background mortality of adult thrips over the 14d confinement trial and 7 d survival trial was estimated using longevity data for adult thrips reared

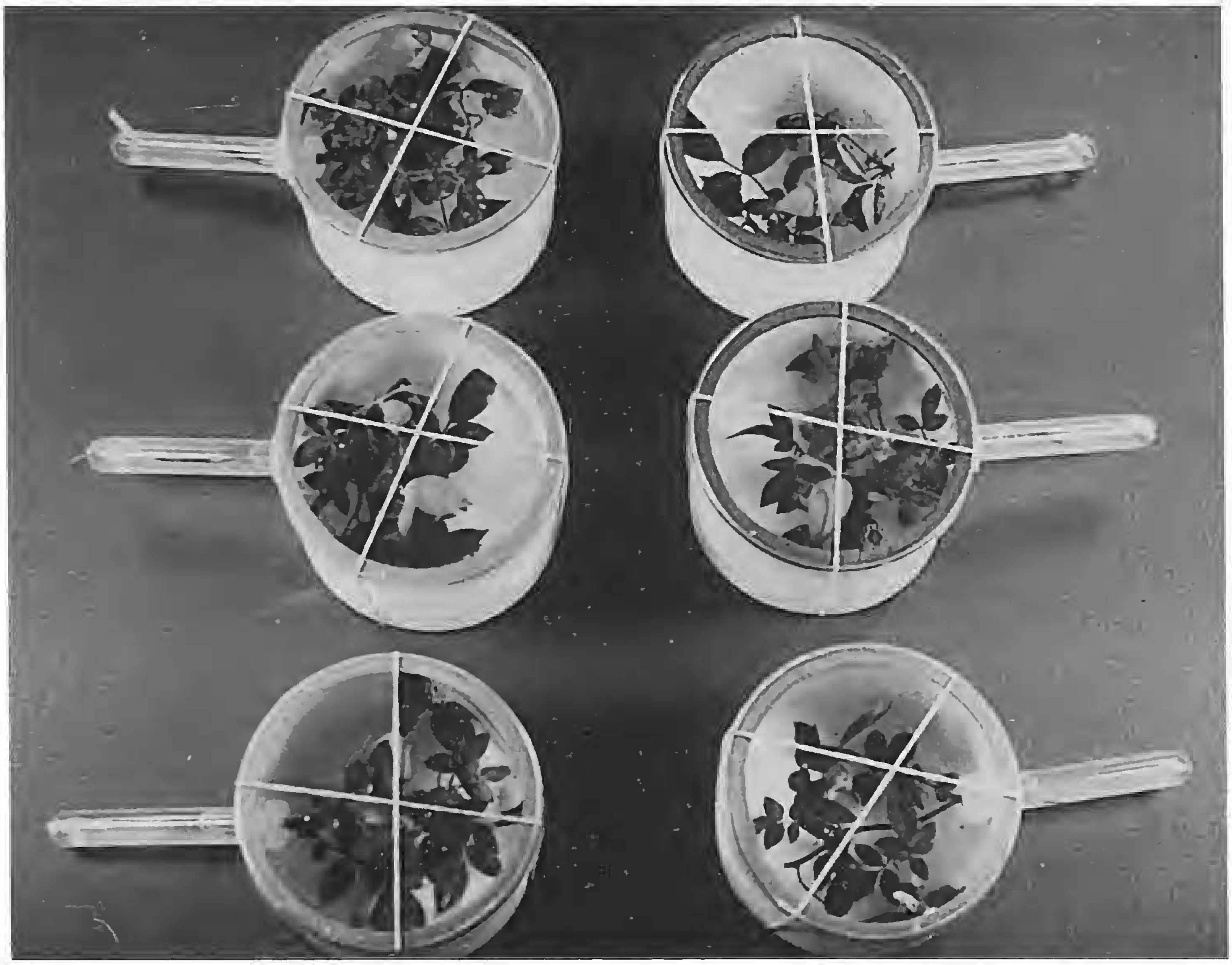

Figure 2. Photograph of experimental arena test units showing rose host material for assays using entomopathogenic fungi against thrips.

on chrysanthemum in environmental chambers from Robb (1989). At 26° C adult longevity is approximately 32 d. Assuming a normally distributed age distribution of adults was obtained from the colony, an expected mortality of approximately 44 percent at 14 d and 22 percent at 7 d was estimated and compared to actual mortality in the arenas. Statistical analyses were performed using t tests.

Results and Discussion

The experimental arenas successfully contained and maintained adult thrips during the 14 d period. For the containment test an average (± SD) of 46.85 ± 1.42 thrips were placed in the arenas and after 14d an average of 45.85 ± 1.29 were recovered demonstrating a 97.9 ± 1.16 percent recovery rate. Adult mortality during this period averaged (± SD) 39.6 ± 4.8 percent which was not significantly different from the expected 44 percent mortality (t = 0.92, df = 6, $P < 0.05$). Thus the results demonstrated thrips could be successfully confined within experimental arenas for extended periods of time without adversely affecting survival rates. The average (± SD) percent adult thrips mortality for the 3 survival test experiments were 12.1 ± 2.9, 11.3 ± 0.75 and 19.2 ± 4.3 percent after 7 d. Thrips mortality was significantly lower than expected for the first two trials (t = 3.41, df = 4, $P < 0.05$; t = 14.26, df = 4, $P < 0.05$) and not significantly different from expected for the third trial (t = 0.65, df = 4, $P >$

0.05). Results demonstrated that efficacy trials could be conducted over a 7 d period without unexpected mortality and at survival rates that would be acceptable for check treatments in laboratory bioassays. Temperatures and relative humidity inside the arenas averaging 26.5° C, and 64 percent respectively, were found to be slightly higher than the environmental chamber of 26° C and 60 percent RH.

Based on these results we have concluded that the arenas may be applicable for conducting bioassays using fungi and other microbial or biorational pesticides against thrips for at least 7 d in length. The depth of the container allows for a full range of observation of insect subjects using dissecting microscopes or through visual observation. We have found that dead insects tend to drop from the plant material and can be easily counted against the white background of the bottom of the container. Mortality as a function of time can therefore be determined without opening the containers. Ventilation of the containers allows for similar internal environmental conditions to outside conditions and the containers are versatile enough to conduct bioassays on many different host plants and host plant parts. The arenas can be constructed rapidly and the materials are inexpensive and readily available. We believe the use of this design may provide a more realistic assessment of mortality under conditions that more closely resemble field conditions.

In addition to thrips, we have successfully used these arenas for conducting efficacy trials against aphids and mites and have begun to evaluate the effects of entomopathogenic fungi on thrips natural enemies. This technique may also have additional experimental applications such as in behavioral studies, survival and longevity studies and experiments examining plant-insect and predator-prey interactions.

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